In the same period Warburg and Christian (1932) had also identified a "yellow ferment" which they characterized as "oxygen-transporting." The prosthetic group (coenzyme) turned out to be flavin mononucleotide (FMN). Warburg and Christian (1938) then identified yet another yellow enzyme whose coenzyme, flavin adenine dinucleotide (FAD), consists of FMN plus adenylic acid. These coenzymes are closely related to vitamin B_2, the vitamin whose deficiency gives rise to pellagra. As with coenzymes I and II, there was considerable confusion for a number of years as to their exact functions and which was the prosthetic group of which flavoprotein. Investigators focusing on the electron transport chain in the 1940s posited two different pathways converging at cytochrome *b*. One coupled the oxidation of most citric acid cycle substrates to the reduction of NAD ($NAD^+ \rightarrow NADH$) and, in turn, its oxidation to the reduction of a flavoprotein. The other pathway, based on the fact that succinic acid was the only citric acid cycle intermediate whose oxidation did not produce NADH, coupled the oxidation of succinic acid directly to the reduction of a flavoprotein. It was thought that each of these flavoproteins was then oxidized, coupled with the reduction of cytochrome *b*. Eventually it was determined that FMN was the flavoprotein on the pathway from NADH to cyctochrome *b*, whereas FAD played a previously-unsuspected role in the second pathway: the oxidation of succinic acid was coupled to the reduction of FAD, which carried the electrons released by that oxidation into the electron transport chain. Finally, the isolation of ubiquinone, coenzyme Q, from beef heart mitochondria (Crane et al., 1957) led to the recognition that it is an additional substance undergoing reversible oxidation-reduction between both FAD and FMN and cytochrome *b* (see Chapter 6).

With the discovery of the steps involved in the citric acid cycle and the electron transport chain, the oxidation pathway from pyruvic acid to water was complete. One aspect of the process, however, was still not addressed – how the energy released in the oxidative reactions was captured in ATP. Hermann Kalckar (1939) and Fritz Lipmann (1939) both demonstrated that the oxidation of intermediaries such as succinate, malate, and pyruvic acid was accompanied by creation of ATP. Because different substrates all resulted in synthesis of ATP, this suggested that the synthesis occurred in conjunction with the oxidation reactions along the respiratory chain linking dehydrogenation of the substrate with molecular oxygen. Such ATP synthesis came to be known as *oxidative phosphorylation* to contrast it with the phosphorylation of ADP directly linked with metabolic intermediates, a process that was referred to as *substrate phosphorylation*. Both Ochoa (1940) and Belitzer and Tsibakowa (1939) showed that in oxidative phosphorylation more than one ATP molecule was formed per atom of oxygen reduced. By focusing on